Review article

Apoptotic cell death: its implications for imaging in the next millennium

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Abstract. Apoptosis, also known as programmed cell death, is an indispensable component of normal human growth and development, immunoregulation and homeostasis. Apoptosis is nature's primary opponent of cell proliferation and growth. Strict coordination of these two phenomena is essential not only in normal physiology and regulation but in the prevention of disease. Programmed cell death causes susceptible cells to undergo a series of stereotypical enzymatic and morphologic changes governed by ubiquitous endogenous biologic machinery encoded by the human genome. Many of these changes can be readily exploited to create macroscopic images using existing technologies such as lipid proton magnetic resonance (MR) spectroscopy, diffusion-weighted MR imaging and radionuclide receptor imaging with radiolabeled annexin V. In this review the cellular phenomenon of apoptotic cell death and the imaging methods which can detect the process in vitro and in vivo are first discussed. Thereafter an outline is provided of the role of apoptosis in the pathophysiology of clinical disorders including stroke, neurodegenerative diseases, pulmonary inflammatory diseases, myocardial ischemia and inflammation, myelodysplastic disorders, organ transplantation, and oncology, in which imaging may play a critical role in diagnosis and patient management. Objective imaging markers of apoptosis may soon become measures of therapeutic success or failure in both current and future treatment paradigms. Since apoptosis is a major factor in many diseases, quantification and monitoring the process could become important in clinical decision making.

Key words: Apoptosis – Programmed cell death – Imaging methods

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Introduction

What is apoptosis?

In the yin and yang of existence, it is no surprise that nature has arranged for the orderly demise of cells that have completed their useful function. The programmed disappearance of cells, apoptosis, occurs under both physiologic and pathologic circumstances. Two examples of physiologic programmed cell death are the disappearance of cells when growth factor stimuli are withdrawn (an integral component of the menstrual cycle) and the deletion of activated immune cells when cells have completed their assigned task.

When apoptosis is dysregulated, i.e., there is too little or too much programmed cell death, disease often ensues [1–6]. Diseases associated with excessive apoptosis include AIDS, neurodegenerative disorders (Alzheimer's disease), myelodysplastic syndromes (aplastic anemia, thalassemia), ischemia/reperfusion injury, progression of heart failure in cardiomyopathies, viral infections (chronic hepatitis), toxin-induced liver disease, organ transplant rejection, graft versus host disease, and adult respiratory distress syndrome (particularly when associated with toxic shock). Diseases associated with too little apoptosis include cancer and autoimmune disorders. Successful treatment of neoplasms with drugs or radiation induces apoptosis in the lesion.

Apoptosis is defined as "the dropping of a petal or leaf from a flower or tree." Kerr et al. [7] originally coined this term to define the series of morphologic changes in adrenal tissue when ACTH is withdrawn. The withdrawal of ACTH causes adrenal cells to fragment into small, variably sized vesicles (apoptotic bodies). Neighboring cells or phagocytes then ingest the apoptotic bodies, resulting in the disappearance of the cell without an inflammatory response. The outcome of this process is the controlled removal of senescent, unwanted, or deleterious cells without inciting an inflammatory reaction that might damage adjacent healthy cells and extracellular matrix (Fig. 1). The process of orderly cell auto-

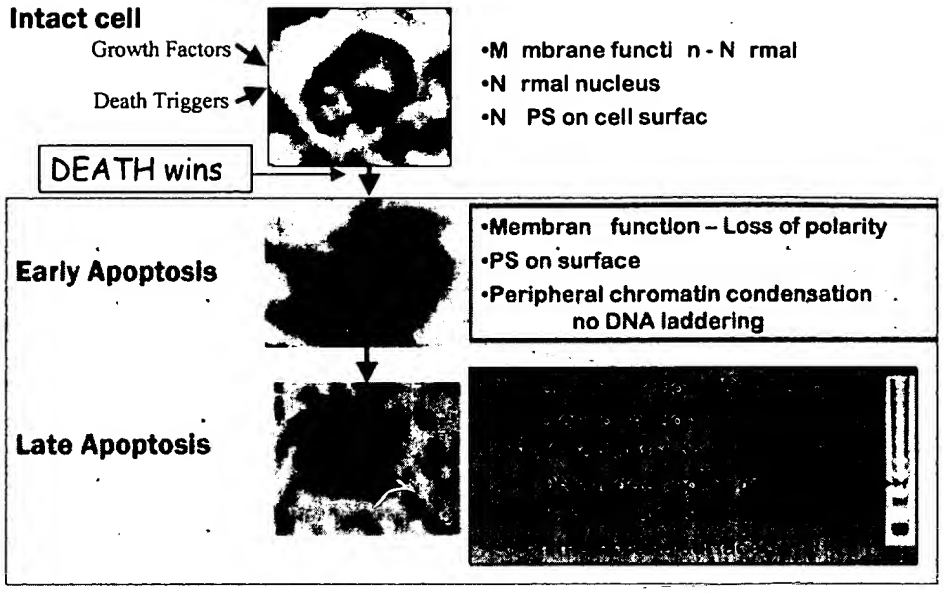

Fig. 1. Sequence of morphologic changes in apoptosis. Initially (*upper panel*) the cell is normal. Following the initiation of apoptosis, chromatin clumping and cytoplasmic condensation occur (*middle panel*). In the late phase of apoptosis (*bottom panel*) the cell begins to form apoptotic bodies and DNA is fragmented with formation for DNA laddering (as indicated on gel electrophoresis)

fragmentation is governed by a cascade of enzymatic activity. Although the original observation suggested that apoptosis is caused by a withdrawal of growth factors, it is now known that a wide array of exogenous or endogenous stimuli [8–12] can trigger the process.

Mechanism of apoptosis

Apoptosis is initiated when there is a disturbance in the local environment of the cell. Cells are constantly bathed in a sea of life-reinforcing stimuli, such as growth factors, and death signals such as tumor necrosis factor. A shift in the balance of these factors with either a decrease in survival factors or a marked increase in death signals can initiate apoptosis. Withdrawal of growth factors, severe mitochondrial damage, attempting mitosis in the presence of irreparable DNA damage, activation of cellular Fas receptors by Fas ligand expressed on activated lymphocytes, hypoxia, heat, cold, chemical injury (chemotherapy), or significant doses of ionizing radiation (causing DNA injury) can result in apoptosis.

Once apoptosis has been triggered, one of several enzymatic cascades is initiated to achieve the orderly destruction of DNA and the dissolution of other cellular elements. A major pathway involves a group of cysteine proteases, the "caspases". Caspase activation precedes morphologic changes. At the time of caspase activation, cells destined for apoptosis appear to signal their neighbors by expressing phosphatidylserine (PS) on the cell surface [13]. PS, along with phosphatidylethanolamine, sphingomyelin, and phosphatidylcholine, is a normal constituent of the cell membrane. In contrast to the other cell membrane constituents, PS is restricted to the inner leaflet of the cell membrane. This constraint on PS distribution is the result of two enzymes, translocase and floppase, which actively pump PS to the inner leaflet and the other lipids out. Activation of caspase is associated with inactivation of these membrane pumps, and activation of an enzyme, scramblase, which equilibrates the membrane lipids on the inner and outer leaflet of the cell membrane – resulting in the rapid appearance of PS on the outer leaflet of the membrane [14, 15]. Once the caspases are activated and PS is expressed, the execution phase of apoptosis occurs. Cellular cytoplasm condenses, nuclear DNA is degraded into 180-kDa pieces and the cell fragments into membrane-covered pieces (each expressing PS on the surface) for phagocytosis [16] (Fig. 1, "Late Apoptosis", bottom panel).

Methods to image apoptosis in vivo

Annexin V

Annexin V [17, 18] is an endogenous human protein which binds to membrane-bound PS with an affinity of about 10^{-9} M. Annexin V, labeled with fluorescein dye, has been used to detect PS expression in studies of apoptosis in hematopoietic cell lines, neurons, fibroblasts, endothelial cells, smooth muscle cells, carcinomas, lymphomas, and all embryonic cell types, as well as nonmammalian plant and insect cells [19]. Annexin V has also been radiolabeled by iodination and coupling to linker molecules such as a diamide dimercaptide (N_2S_2) [20] or hydrazino nicotinamide [20]. The universality of annexin V binding to various cell lines is due to the composition of the phosphoserine head group of PS (the site of annexin V binding), which is identical in all multicellular organisms. In vitro cell binding studies demonstrate a 20-fold increase in annexin concentration in cells undergoing apoptosis compared with control cells.

Table 1. The biodistribution of annexin V in rats

	10 min (n=6)	30 min (n=7)	1 h (n=7)	3 h (n=6)
A) % I.D./organ[a]				
Brain	0.0681±0.0202	0.0197±0.0035	0.0149±0.003	0.0093±0.0009
Lungs	2.611±0.367	1.558±0.191	1.000±0.30	0.821±0.085
Heart	0.372±0.0726	0.150±0.048	0.135±0.033	0.134±0.0165
Liver	20.8±1.179	15.8±2.67	21.6±4.12	17.97±1.36
Spleen	4.64±0.459	4.08±1.089	4.75±1.22	4.12±0.571
Kidneys	29.2±6.05	33.5±8.02	45.6±5.48	47.4±2.90
Stomach	0.25±0.056	0.376±0.038	0.51±0.165	0.38±0.138
B) % I.D./gram[b]				
Small intestine	0.144±0.066	0.114±0.048	0.128±0.059	0.129±0.011
Colon	0.082±0.066	0.054±0.013	0.052±0.014	0.082±0.039
Skeletal musc.	0.035±0.009	0.024±0.007	0.026±0.008	0.022±0.002
Bone marrow	2.13±0.52	1.55±0.72	2.083±0.57	1.98±0.374
Blood	1.38±0.34	0.306±0.098	0.215±0.090	0.107±0.013

Mean values±standard error for tissue and organ samples from control Sprague-Dawley rats 10 min, 30 min, 1 h and 3 h after injection of 25 μg/kg of ^{99m}Tc-HYNIC-annexin V (500–600 μCi/animal) labeled using a ^{99m}Tc-tricine conjugate method injected via tail vein. Samples were weighed and placed in 3-ml tubes and counted

[a] Mean percentage of injected dose (%I.D.) per organ (% I.D./organ)

[b] Mean percentage of injected dose per gram of tissue (%I.D./g)

Tait and colleagues utilized radiolabeled annexin V to detect increased PS expression which occurs when platelets are activated in acute thrombosis [21]. Blankenberg and colleagues utilized radiolabeled annexin to detect apoptosis in vivo in animal models of transplant rejection, hypoxic cerebral injury, tumor therapy and Fas induced apoptosis [22, 23].

Following intravenous administration of radiolabeled annexin V, the agent is cleared from the blood with a half-time of less than 5 min, and is concentrated primarily in the kidneys and to a lesser degree in the liver. A summary of annexin biodistribution in rodents is presented in Table 1. Apoptosis induced by stimulation of the Fas/Fas ligand system, transplant rejection, or chemotherapy in tumor-bearing animals has been visualized with technetium-99m labeled annexin imaging.

The minimal cell mass required for successful detection of apoptosis by in vivo imaging is not known. However, in studies of heart transplant rejection by Vriens et al., histologic evidence of apoptosis was present in <10% of cells at the time when annexin imaging demonstrated remarkable focal localization in the transplant (see later discussion of heart transplant rejection). It is likely that single-photon imaging will require significant apoptosis (in at least 10% of cells) to detect the process in a small mass of tissue (~2–3 g). If the extent of the lesion involves a larger mass of tissue, such as the major portion of an organ, less extensive apoptosis will be detectable. It is likely that the contrast of annexin images will be enhanced if the protein is labeled with fluorine-18 and data recorded with positron tomography.

In addition to radionuclide imaging with radiolabeled annexin, other imaging modalities, such as magnetic resonance (MR), have been utilized to detect apoptosis in-vivo.

MR lipid spectroscopy and diffusion-weighted MR imaging

Since 1982 numerous in vitro and in vivo MR studies have documented the presence of a narrow and intense resonance in the 1.3-ppm region of the lipid proton spectra from cultured tumor, embryonic, and stimulated lymphocyte cell lines and solid, experimental and human tumors [24, 25]. Of note, this resonance has been found to increase in solid tumors following treatment and the induction of apoptotic cell death. A rise in the 1.3-ppm resonance has also been observed acutely after hypoxic-ischemic injury of the cerebrum [26] and cerebral diffuse axonal injury due to child abuse [27] and has been found to be of prognostic significance. This resonance has now been recognized as representing the methylene proton (-CH_2-) of mobile neutral lipid fatty acid chains within the plasma membrane bilayer in vitro [23, 24] and cytoplasmic vesicles in vivo [28, 29].

Coincident with the lipid rearrangements that permit both radiolabeled annexin V and proton lipid MR spectroscopic imaging of apoptotic cells and tissues is the shrinkage of a cell's cytoplasmic volume. There is concurrent increased cytoplasmic microviscosity and restriction of water motion [30]. This apoptotic phenomenon can be imaged by MR using diffusion weighted imaging (DWI), which tags and follows the motion (diffusion or ADC = the average diffusion coefficient) of individual water molecules [31]. The use of DWI to detect and

^{99m}Tc-Annexin ^{99m}Tc-DTPA

Fig. 2. Hypoxic brain injury due to transient occlusion of the right middle cerebral artery. Seven hours following occlusion/reperfusion, pinhole vertex view images of the head in intact adult rats were obtained after injection of annexin (*left panel*) to identify apoptosis and DTPA (*right panel*) to identify areas of blood-brain barrier breakdown. There is no loss of blood-brain barrier integrity, but annexin uptake is seen in the right hemisphere (*arrow*). The nose is oriented cephalad on images

track apoptotic cell death in vivo is only beginning to be studied and its ultimate clinical applicability, particularly outside the nervous system, remains to be defined.

The following sections describe some specific diseases where apoptosis is known to play a significant role, and where imaging the process may be helpful in clinical decision making.

Central nervous system

Stroke

Hypoxic-ischemic injury (HII) in adults (stroke, multi-infarct dementia) with delayed loss of gray and white matter is due to apoptosis [32]. Blankenberg et al. [33] have shown that moderate to severe injury, without evidence of blood-brain barrier breakdown, is associated with remarkable focal radiolabeled annexin uptake in an adult rat model of ischemic/reperfusion hemispheric injury (Fig. 2). Because the actual cell loss in these patients is gradual (delayed with respect to the insult) there may be a therapeutic window to inhibit (or reverse) early apoptosis with pharmacologic blockade. Imaging with radiolabeled annexin V may be helpful in addressing this clinical problem since annexin V has virtually no background uptake in the brain.

Ischemia/reperfusion injury in preterm/term infants

HII in preterm infants is a major cause of cerebral palsy. Because of the immaturity of the centrifugal cerebrovascular circulation, full-term and preterm neonates manifest HII in a different fashion than adults [34, 35]. Preterm infants tend to suffer watershed ischemic injury in a periventricular distribution (periventricular leukomalacia or PVL) while term infants develop disease of their subcortical white matter. The delayed cell loss of these injuries is mediated by apoptosis [36]. In experimental animals ^{99m}Tc-annexin imaging has clearly delineated these lesions in the absence of blood-brain barrier breakdown.

Traditional imaging techniques do not provide the specificity needed to identify neonates at risk for development of cerebral palsy (which is usually diagnosed at 2–3 years of age) who might benefit from these novel treatments in the first several days (or hours) of life. Reversible abnormalities seen by DWI MR associated with mild transient hemispheric HII resulted in multifocal regions of abnormal annexin V cerebral and cerebellar uptake in a neonatal rabbit model of global hypoxia [37]. Histology of these neonatal rabbit brains demonstrated a correlation between radiolabeled annexin V uptake and subtle scattered ischemic changes in the hippocampus periventricular/subcortical white matter. In the future, neonates who are the products of a difficult labor or delivery could be assessed for suspected HII damage with annexin V imaging to identify patients who might benefit from therapy.

AIDS dementia/Alzheimer's disease

Neuronal and glial cell apoptosis also occurs in acquired immune deficiency syndrome, in encephalitis with or without AIDS-related dementia and in neurodegenerative disorders, such as Alzheimer's and Parkinson's disease [38]. The gradual loss of white and gray matter in Alzheimer's disease is primarily due to apoptosis. Imaging with radiolabeled annexin V could be helpful in the diagnosis and therapeutic management of this diverse group of patients.

Lung disease

High-resolution computed tomography (HRCT) of the chest has enhanced the detection and characterization of disease processes affecting the interstitium of the lung [39]. Diseases such as idiopathic pulmonary fibrosis, desquamative interstitial pneumonitis, *Pneumocystis carinii* pneumonia, lymphocytic interstitial pneumonia, fibrosis in collagen vascular diseases, sarcoidosis, drug or allergic reactions, bronchiolitis obliterans, and bronchiolitis obliterans with organizing pneumonia [40] can be readily identified. These entities, however, all involve cell-mediated inflammation and activation of T lymphocytes, at least in the acute/subacute settings. Although HRCT provides characteristic images, often obviating the need for confirmatory lung biopsy, HRCT cannot

quantify the degree of inflammation. The degree of inflammation is important to define the best management of the process. Without specific information about inflammation, clinicians manage these patients with pulmonary function tests, bronchoalveolar lavage, and serial chest radiography.

Radiolabeled annexin V imaging of the chest may be of value to quantify cell-mediated inflammation and apoptosis in the lungs. In a rat model of cell-mediated lung apoptosis, acute lung transplant rejection, radiolabeled annexin V localization was more sensitive than CT scanning in detecting the process. Other circumstances where apoptosis imaging may play a role include: adult respiratory distress syndrome, which is in part induced by the release of tumor necrosis factor, a potent inducer of apoptotic alveolar cell death; bronchopulmonary dysplasia; neonates with pulmonary oxygen toxicity; cystic fibrosis; and asthma. In each case, the extent of apoptosis will indicate the effectiveness of therapy – if apoptosis is extensive, therapy is not controlling the process.

Heart

Myocardial infarction and reperfusion injury

Like the brain, cardiomyocytes undergo apoptosis in response to hypoxic-ischemic insults that are insufficient to induce frank necrosis [41] [e.g., the peripheral (penumbral) regions of an infarct or following reperfusion of an infarct]. Interestingly, the heart also displays cytoplasmic lipid droplets, the presence of which confers a poorer prognosis in animal models of myocardial ischemia [42]. It is clear that agents which selectively inhibit activation of the apoptotic enzymatic cascade decrease the degree of "infarction" in both the heart and brain in response to an ischemic insult [43, 44]. Preliminary studies of myocardial annexin localization in rats with acute myocardial infarction suggest that apoptosis plays a major role in cell death following acute coronary occlusion (S. Hasegawa and T. Nishimura, personal communication).

Coronary disease and atherosclerosis

Recent biochemical evidence strongly suggests that activated monocytes/macrophages attracted by local vascular endothelial damage and complement activation infiltrate the arterial vascular wall [45]. With continued inflammation there is deposition of lipid at the site of injury, formation of foam cells (lipid-laden macrophages), and formation of an atheroma. Within the plaque there is significant apoptosis involving the monocytes and macrophages infiltrating the lesion, smooth muscle cells at the base of the lesion, and, in unstable plaque, the endothelial cells forming the cap of the plaque. This last event is particularly dangerous as the apoptotic endothelial cells expressing PS on their surface serve as thrombogenic foci.

Fig. 3. Two rats with heterotopic hearts transplanted into the abdomen, below the kidneys. One hour following injection of ^{99m}Tc-annexin, anesthetized animals were placed prone on a high-resolution parallel-hole collimator. Images of the syngeneic (*left*) and allogeneic (*right*) transplants were recorded on the fourth day after transplant. The bright area in the mid abdomen is the transplant undergoing rejection/apoptosis in the allogeneic animal. Some annexin is seen in the baldder of both animals

It is unclear whether external imaging with ^{99m}Tc-annexin V will have sufficient resolution to define these lesions; however, if an intravascular probe were available such lesions might be readily identified. An alternative strategy may involve the use of radiolabeled MCP-1 (monocyte chemotactic peptide), which has a molecular weight of approximately 13,000 [46]. MCP-1 selectively binds to "activated" macrophages and has shown promise in detecting mononuclear infiltration in the subendothelial layers of mechanically traumatized arterial vessels. As activated monocytes/macrophages are intimately involved in chronic inflammation, MCP-1 may be useful as a marker of infectious or noninfectious granulomatous inflammation throughout the body [47].

Myocarditis/cardiomyopathies

Apoptotic cell death plays a major role in viral and autoimmune myocarditis and nonischemic cardiomyopathies [7]. Acute transplant rejection is primarily an immune event mediated by alloreactive T lymphocytes. In a rat model of acute cardiac transplant rejection, serial imaging with ^{99m}Tc-annexin V successfully detected rejection [48] with an accuracy exceeding that of TUNEL staining of biopsy specimens (Fig. 3). Furthermore, when immunosuppressive therapy with cyclosporine was initiated, the decrease in rejection was mirrored by a marked decrease in ^{99m}Tc-annexin localization (Fig. 4). In light of this experience with transplant rejection, annexin V imaging may also be helpful in the detection of apoptosis due to virally induced cardiomyopathies or other autoimmune diseases that affect the heart such as systemic lupus erythematosus, rheumatic fever, and Kawasaki's disease.

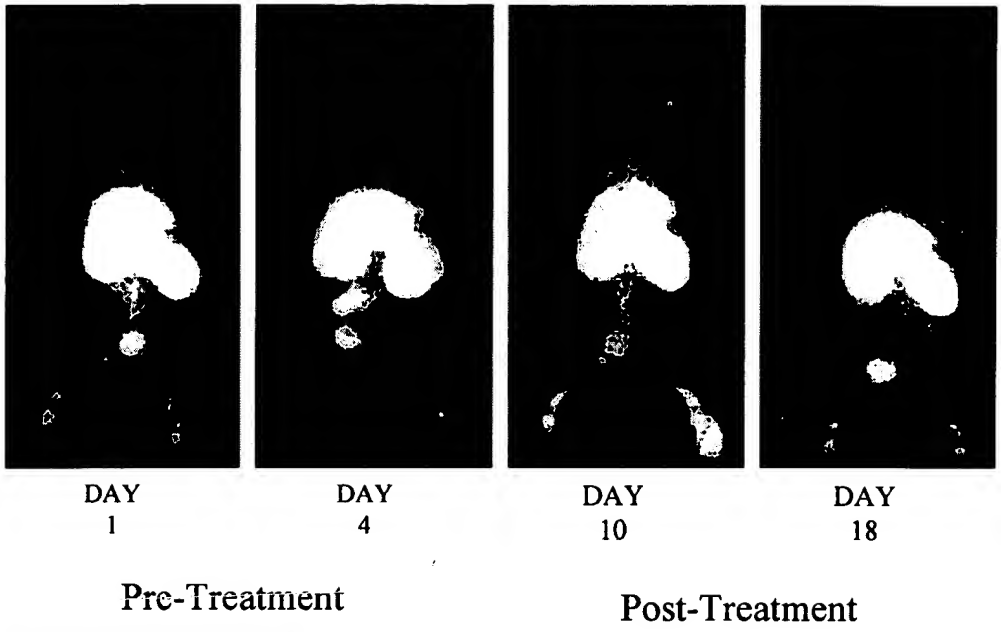

Fig. 4. Annexin imaging in acute cardiac transplant rejection before and after cyclosporine therapy. The four panels depict whole body images on days 1, 4, 10, and 18 after transplantation (each image was recorded 1 h after ^{99m}Tc-annexin injection). On day 1 there is no annexin imaging evidence of apoptosis. The image on day 4 demonstrates ^{99m}Tc-annexin localization in the transplanted heart. Treatment with cyclosporin commenced on day 5, and by day 10 the annexin localization has diminished. By day 18, annexin localization has declined further

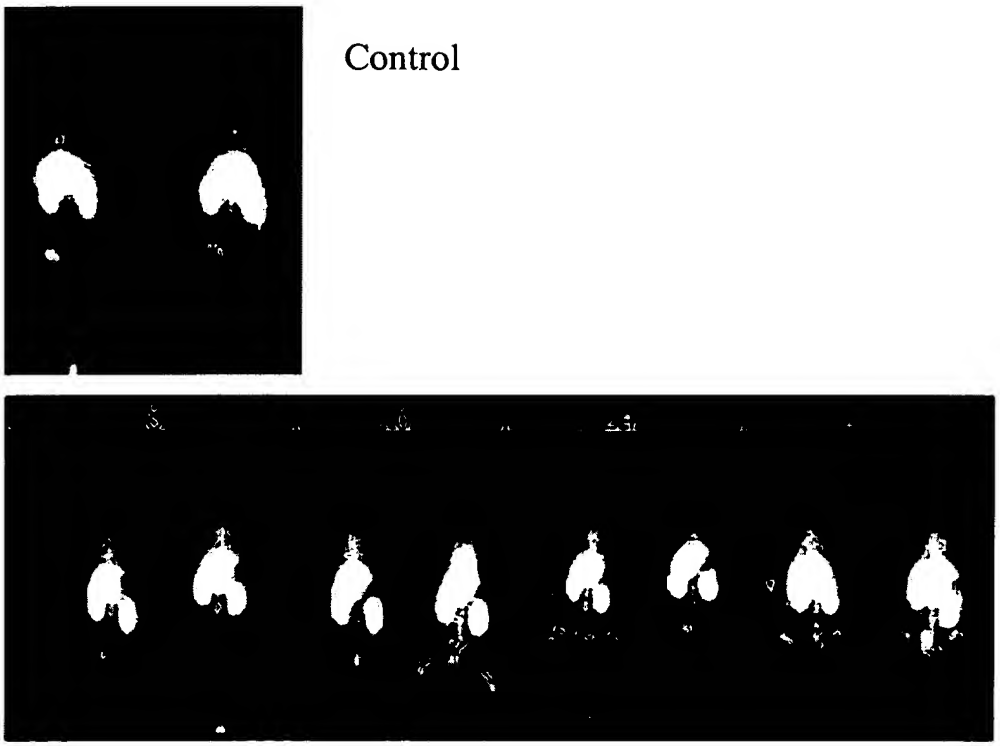

Fig. 5. Whole body posterior images in rats 1 h following tail vein injection of 1 mCi of radiolabeled annexin V before and after treatment with 100 mg/kg of cyclophosphamide administered intraperitoneally to ablate the bone marrow. One day later the animals were imaged. Control animals are shown in the *top panels* and rats that had received cyclophosphamide 8–72 h before annexin imaging are shown in the *bottom panels*. The vertebral and peripheral marrow show increased localization (350%) following cyclophosphamide-induced apoptosis

Bone marrow diseases

β-thalassemia, sickle-cell disease, aplastic anemia (myelofibrosis), parvoviral infection (and many other viral infections), autoimmune diseases, toxins, radiation, and chemotherapy cause significant apoptosis in the bone marrow [7]. Bone marrow aspiration is required to determine the degree of disease involvement of the bone marrow [49]. We have shown that cyclophosphamide (a potent bone marrow suppressive and antitumor agent) treatment of otherwise normal adult rats induces a marked increase in bone marrow uptake of annexin V as compared with controls (Fig. 5). Based on this experimental observation, it is likely that ongoing apoptotic bone marrow disease processes such as aplastic anemia may be detected with annexin imaging.

Joint diseases

Autoimmune, crystal depositional, and idiopathic arthropathies all appear to be intimately linked to apoptotic cell death of the synovial tissues [7]. Other than symptomatic improvement there are few objective measures with which to guide anti-inflammatory therapy. Efforts are underway to characterize and quantify the quality of hyaline cartilage using a number of MR techniques [50]. Annexin V (and probably MCP-1) imaging may in fact provide an objective measure of disease severity and anti-inflammatory treatment response. Additionally, the gradual loosening and failure of joint prostheses appears to be directly linked to the apoptotic cell death of macrophages which are activated in response to foreign debris [51]. Annexin V imaging may be useful in the management of these patients as well.

Organ transplantation

Annexin V can identify acute heart, lung, and liver transplant rejection in vivo [52]. The majority of transplant recipients, however, do not have acute graft rejection, but instead suffer multiple clinically manifest and subclinical episodes of rejection. A combination of smoldering inflammation and a low level of apoptosis are some of the key findings in chronic rejection. The inflammation and apoptosis are primarily manifest as accelerated vascular disease (atherosclerosis) in the graft. Graft vasculopathy is a process that occurs over years, characterized by perivascular mononuclear cell infiltration and apoptotic cell death. The chronicity of the process may be associated with such a low level of apoptosis that the process cannot be detected by annexin V imaging.

Oncology

The degree of cytoreduction in response to antitumor treatment(s) directly correlates with overall disease response, disease-free interval, and ultimate survival in a number of malignancies [53]. Depending on the type of malignancy the only measures of cytoreduction are gross shrinkage or disappearance of tumor as seen by anatomical imaging with ultrasound, CT or MR imaging. Recently, there has been a renewed interest in using proton lipid MR spectroscopy and DWI MR to assess the early treatment response of tumors [26–28]. Unfortunately, these techniques cannot give information except for regional areas of tumor involvement, particularly during induction therapy, when patients can be quite ill. In addition DWI MR detection of tumoral apoptosis relies on the observable shrinkage of the cell cytoplasm during tumor cell death. However, many tumors respond to chemotherapeutic agents which specifically attack DNA, such as doxorubicin (adriamycin), by cell swelling, not

Treated tumor in left thigh

Fig. 6. Annexin V detection of necrotic versus apoptotic tumor cell death in flank tumors of mice following chemotherapy. The animal on the *left* demonstrated a greater than 363% increase in left flank 38C13 murine B cell lymphoma annexin V uptake 20 h after treatment with 100 mg/kg of cyclophosphamide (i.p.) compared with untreated flank lymphoma (animal on right)

cell shrinkage. Therefore, DWI MR may give misleading results in vivo. This cell swelling is due to a metabolic cell death initiated by massive activation of a normally quiescent nuclear enzyme called poly-ADP-ribose polymerase (PARP or PARS) [54]. PARP is generally activated in the later stages of apoptosis and helps induce fragmentation of a cell's DNA. Direct DNA damage by ionizing radiation, or radical ion formation by agents such as doxorubicin or HII [55] in some circumstances can induce massive direct activation of PARP. Activated PARP utilizes NAD to form poly-ADP ribose polymers. To replace lost NAD stores a cell utilizes ATP and if PARP activation is of sufficient intensity, virtually all ATP stores of a cell will be rapidly depleted. A cell depleted of ATP will simply swell and lose membrane integrity (necrosis). In an experimental model of lymphoma treated with cyclophosphamide, therapy-induced apoptosis is readily visible with ^{99m}Tc-annexin V imaging (Fig. 6). Despite these different mechanisms of cell death, annexin V imaging will demonstrate the process because annexin V binds to cells expressing PS on the outer cell surface (apoptosis) or, alternatively, annexin can gain access to inner plasma membrane leaflet PS after the onset of irreversible membrane failure (PARP-mediated cell death).

Summary

Medicine is ready to proceed beyond the era where therapy is guided by patient symptoms or complaints. Objective markers of therapeutic success are necessary to maximize the benefit of new treatment paradigms. As molecular biology provides insight into the pathophysiology of disease and ushers in therapies for disorders that are now untreatable, characterization of disease will play a significant role in clinical decision making. Since apoptosis is a major factor in many diseases, quantifying the process can play a role in patient care. In patients with neoplasms, for example, imaging the induction of apoptosis following the first dose of an effective treatment would play a major role in treatment selection (in addition to the savings of time and money). Similarly, in patients with connective tissue disorders, quantifying the reduction of apoptosis following introduction of successful therapy would be useful. Imaging apoptosis is likely to be just the beginning. Detecting vascular growth factor expression, identifying cellular response to chemotactic signals, and quantifying tissue rates of cell division will also be added to the armamentarium in the near future. These imaging techniques will make the imaging of function as important in the twenty-first century as imaging anatomy is in the twentieth.

To conclude, potential medical imaging strategies using ^{99m}Tc-annexin V imaging are summarized below:

- Identification of cerebral hypoxic-ischemic brain injury. Since apotosis can be reversed in the early phases of the process, annexin imaging can play a major role in the selection of therapy in the first several hours following stroke in adults, or following difficult delivery in the newborn.
- Screening of patients at risk or with early signs of neurodegenerative diseases to offer objective evidence of the disease process.
- Monitoring immunocompromised patients for pulmonary infections such as *Pneumocystis carnii*, cytomegalovirus, and aspergillosis.
- Screening and following therapy for bronchiolitis obliterans with or without organizing pneumonia (acute and chronic lung transplant rejection), and other interstitial processes such as lymphocytic interstitial pneumonia, desquamative interstitial pneumonitis, sarcoid, and other granulomatous infectious or noninfectious pulmonary diseases.
- Detection of myocardial apoptotic cell death following repeated episodes of ischemia or myocarditis.
- Defining the site and extent of apoptosis in the bone marrow in myelofibrosis, thalassemia, sickle cell anemia (aplastic crisis), and the side-effects of chemotherapy/radiation.
- Monitoring of bone marrow transplant recipients for graft versus host disease.
- Detection and monitoring the efficacy of therapy for joint diseases/inflammation in juvenile- and adult-onset rheumatoid arthritis and muscular/soft tissue degenerative diseases such as scleroderma, polymyositis/dermatomyositis, and Duchenne's muscular dystrophy.
- Earlier assessment of the efficacy of cytoreductive therapy for leukemia, lymphoma, sarcomas, and unresectable primary or bulky metastatic disease to identify tumor response within 24 hours after starting therapy.
- Monitoring the effects of gene therapy designed to either inhibit or induce apoptotic cell death in a particular anatomic location or organ.

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